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SARCOPLASMIC RETICULUM IN THE ADDUCTOR MUSCLES OF A BERMUDA SCALLOP: COMPARISON OF SMOOTH *VERSUS* CROSS-STRIATED PORTIONS

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Abstract

The adductor muscle of the Bermuda scallop, *Pecten ziczac*, is composed of two different types of muscle: cross-striated and smooth. The major portion consists of ribbon-shaped cross-striated muscle cells averaging about ten μm by 1.5 μm in cross section. Each cell contains only one myofibril. In some of the wider cells, an extra sarcomere sometimes is inserted in the lateral part of the myofibril creating a vernier. The individual striated muscle cells do not span the entire length of the adductor but are connected at their ends via junctions similar to intercalated discs. The minor portion of the adductor muscle consists of smooth muscle cells which are fusiform averaging 6 μm in diameter. There are no specialized cell surface invaginations in either muscle type that correspond to a T-tubule or caveolae system. The sarcoplasmic reticulum in both muscles is confined to the area just beneath the cell surface. In both muscle types, the sarcoplasmic reticulum systems have distended vesicles connected to the cell membrane via surface couplings. These vesicles are interconnected by one to seven tubular elements of the sarcoplasmic reticulum to form a closed and continuous system. The tubular elements of the sarcoplasmic reticulum in the cross-striated muscle are fairly uniform in bore, 40 nm in diameter, as compared to the irregular bore in smooth muscle, 15–40 nm. The striated muscle has twice as much of its surface covered with sarcoplasmic reticulum as does the smooth muscle. Moreover, the striated muscle cells have about 4% of their cross-sectional area devoted to sarcoplasmic reticulum while the smooth muscle cells have only 0.5%. This eight-fold difference in the amount of sarcoplasmic reticulum in the striated muscle is consistent with its reported 50-fold faster contraction rate and 128-fold faster relaxation time over that of the scallop adductor smooth muscle.

Introduction

The adductor muscle bundle in the scallop consists of two different types of muscle. The larger part (about 90%) is translucent, is composed of cross-striated muscle cells (Marceau, 1909), and is responsible for the swimming action of the scallop (von Buddenbrock, 1911). The smaller, white part of the adductor consists of smooth muscle cells responsible for keeping the shells closed for long periods of time with low consumption of energy (von Buddenbrock, 1911) and has been termed a "catch" muscle (von Üxküll, 1912; Bayliss *et al.*, 1930). There is as yet no satisfactory explanation as to how the catch mechanism works (Johnson *et al.*, 1959; Lowy and Millman, 1963; Rüegg, 1971; Twarog, 1975). The scallop cross-

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striated adductor contracts at a rate that is fifty times that of the smooth adductor and relaxes after a contraction at a rate that is 128 times faster than the smooth part of the adductor (Prosser, 1973). The present study examines the structure of the sarcoplasmic reticulum in the cross-striated and catch muscles and provides an analysis of the amount and distribution of the sarcoplasmic reticulum in the two parts of the adductor. The results provide a morphological basis for the different contraction and relaxation rates of the two muscle types.

Materials and Methods

Bermuda scallops, *Pecten ziczac,* were collected from Harrington Sound and kept in aquaria at the Biological Station until used. The scallops were taken out of the water and gently shaken to remove the sea water inside the shell and then placed in sea water (22°C) containing 4% glutaraldehyde (Polysciences, Inc., Warrington, PA). The pH of the glutaraldehyde-sea water was 7.5 and was not adjusted. After 4 to 24 hours the solution was changed and fresh fixative was added.

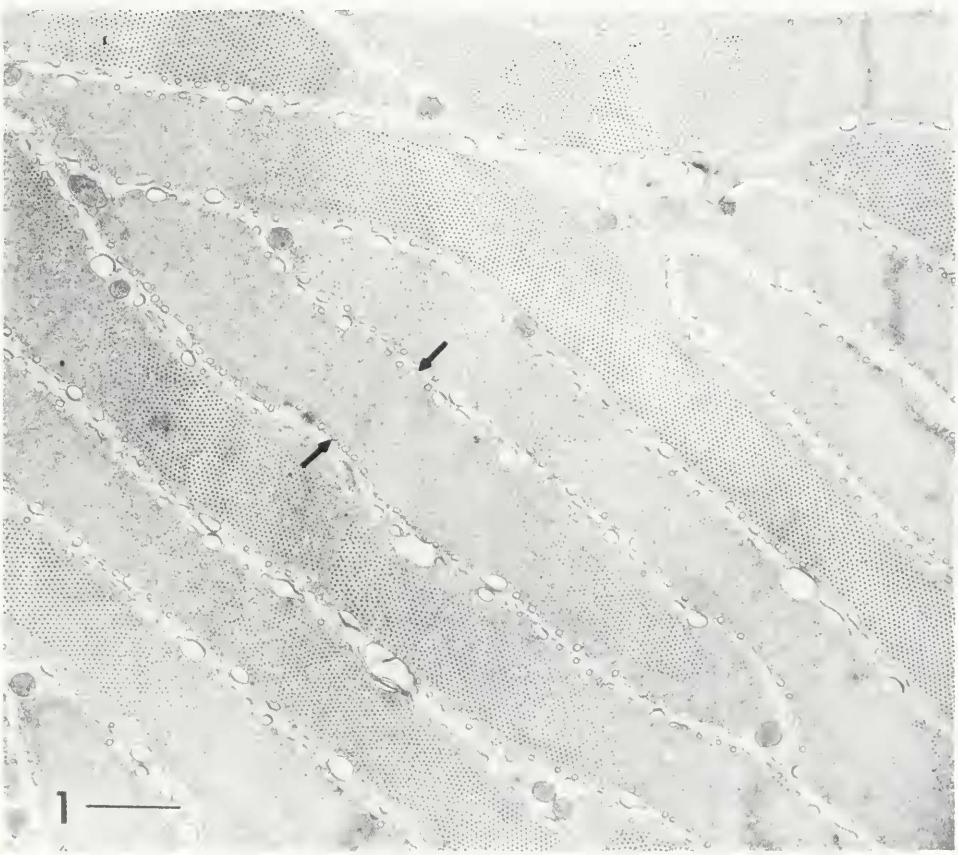

FIGURE 1. Cross-section of striated muscle reveals ribbon shape of myofibrils. Sarcoplasmic reticulum (arrows) are localized just under the cell surface. Scale = 1 μm.

FIGURE 2. Longitudinal section of striated muscle illustrating the narrow part of the ribbon shaped cells. Each cell contains one myofibril. Scale = 1 μm.

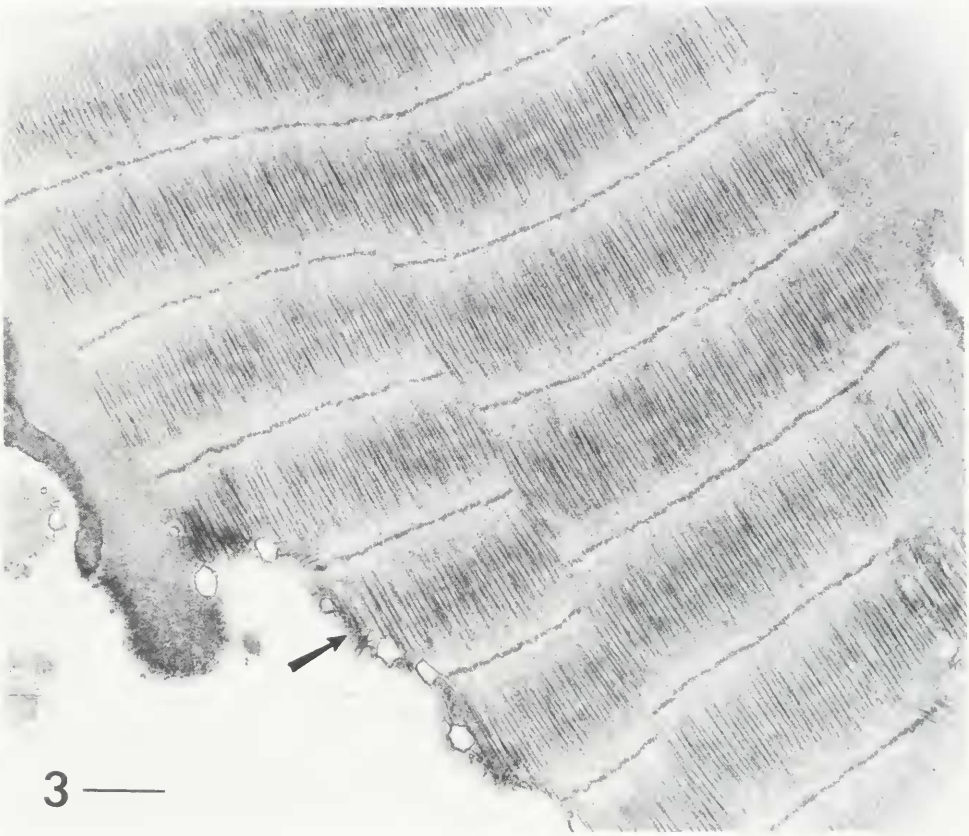

FIGURE 3. Demonstration of a vernier in which an extra sarcomere (arrow) is inserted on the left side of the myofibril. This longitudinal section illustrates the wide part of the ribbon-shaped cell. Scale = 1 μm.

The total time in glutaraldehyde fixative was one to two days. The shell was removed from the muscle and the two parts of the adductor, smooth and cross-striated, were separated from one another. Small pieces were cut from each muscle bundle and washed several times over a four to six hour period with filtered sea water and then fixed in osmium tetroxide (1% in 0.1 *M* phosphate buffer, pH 6.0), in the cold (4°C). The muscle was rinsed at room temperature many times over a one hour period with distilled water, stained *en bloc* with an aqueous solution of uranyl acetate (0.25%) for two to four hours and then rinsed several times with distilled water before being dehydrated in an ethyl alcohol series and embedded in Epon (Luft, 1961). Thin sections were stained with freshly filtered 4% aqueous uranyl acetate solution, followed by lead citrate (Reynolds, 1963) and examined with a Philips EM 201 electron microscope (provided on loan to the Bermuda Biological Station courtesy of Philips Inc. and Lico, Inc.).

Measurement of the amount of sarcoplasmic reticulum was calculated from electron micrographs of cross-sections of the smooth and striated muscle cells. Thirty micrographs, each a cross-section through a different cell (15 smooth and 15 striated), were printed at the same magnification on 11″ × 14″ photographic paper. Each cell in the cross-section was then cut out of the paper along the outer cell

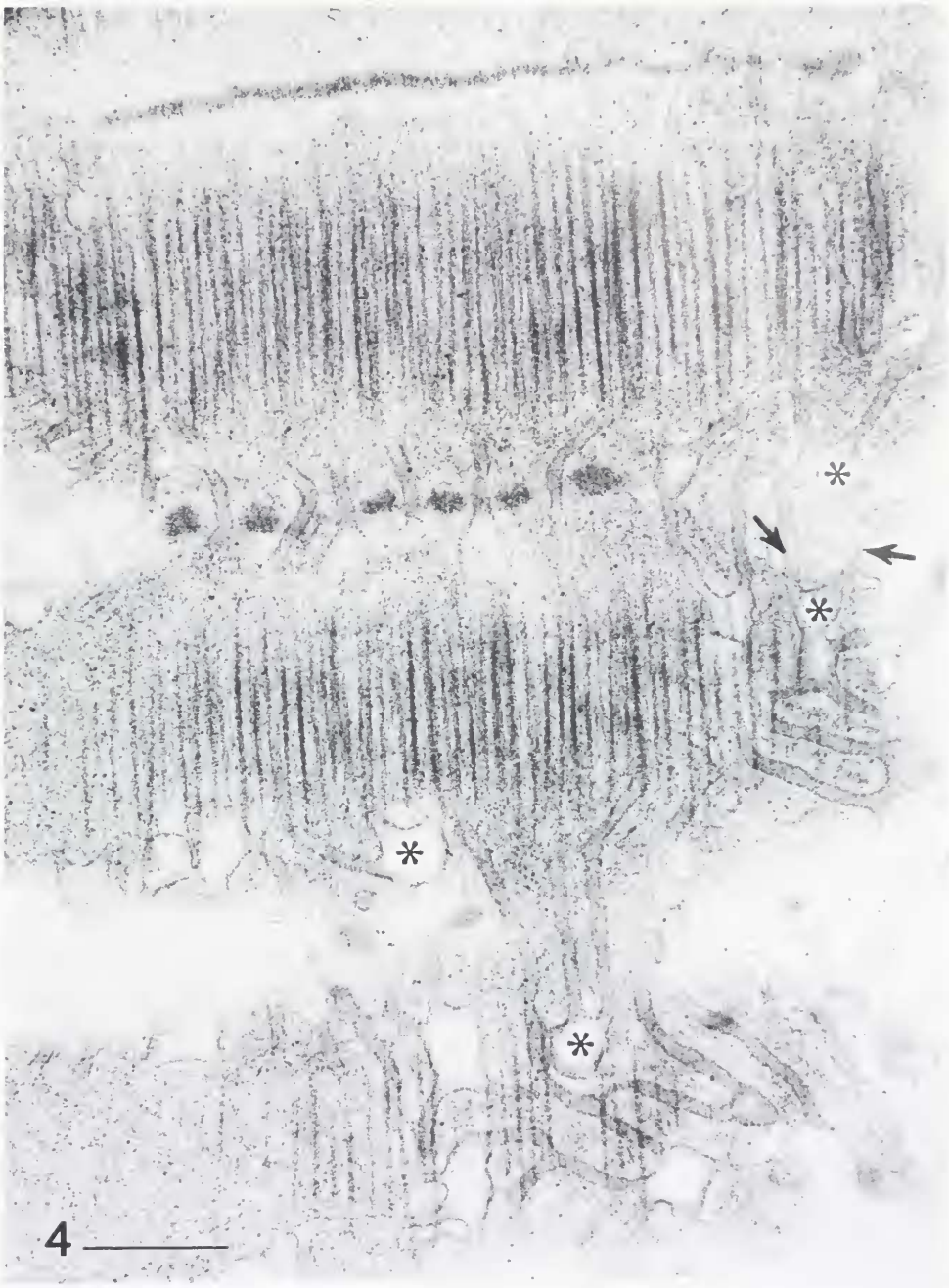

FIGURE 4. The terminal cisternae (asterisks) of the sarcoplasmic reticulum are interconnected by a series of tubular elements (arrows). Scale = 0.5 μm.

membrane and the cell profile weighed. An Exacto knife was then used to cut out the elements of the sarcomplasmic reticulum from beneath the outer membrane of the cell profile, and the sarcoplasmic reticulum profiles were then weighed. The

ratio of sarcoplasmic reticulum weight to cell weight was considered to represent the percent of cross-sectional areas of the cell occupied by sarcoplasmic reticulum.

Results

Cross-striated adductor

The cross-striated muscle cells of the Bermuda scallop, *Pecten ziczac,* are ribbon-shaped, ranging from 0.9 to 2.0 μm (ave. = 1.5 μm) in thickness and from 3 to 20 μm (ave. = 10 μm) in width (Figs. 1, 2). They do not run the entire length of the adductor but are connected via junctions between the terminal Z-bands. The junctions are staggered along the length and width of a cell to form interdigitating boundaries similar to those found in the intercalated discs in cardiac muscles. Each cell consists of one myofibril and in some cells an extra sarcomere is inserted laterally creating a vernier effect (Fig. 3). There seems to be only one elongated nucleus per cell and it is located at the periphery of the cell.

The sarcoplasmic reticulum is confined to the area just beneath the cell surface and can be observed most clearly in tangential sections cut just below the plasmalemma (Figs. 4, 5). There are no transverse tubules or sarcoplasmic reticulum in the interior of the cell. The sarcoplasmic reticulum consists of two units: flattened vesicles and tubes linking the vesicles to form a closed system. The vesicles are about 0.16 μm in width and up to 0.28 μm in length and are interlinked by 2–7 tubular elements, all of which have a uniform bore of about 40 nm. Infrequently, one tube terminates in another tube and in a few cases vesicles fuse to each other directly. The sarcoplasmic reticulum vesicles and tubes are scattered all along the surface of the sarcomere with no periodic arrangement at a particular band of the sarcomere. In cross-sections, surface couplings between the vesicles and the cell surface were observed (Fig. 5), but only occasionally were couplings seen between the smooth tubes and the cell surface. Both vesicles and tubes had fine granular material associated with their luminal wall membrane. Together, the vesicles and tubes comprising the sarcoplasmic reticulum accounted for about 4% (average of 15 cells) of the non-nuclear cross-sectional area of the cross-striated adductor cell.

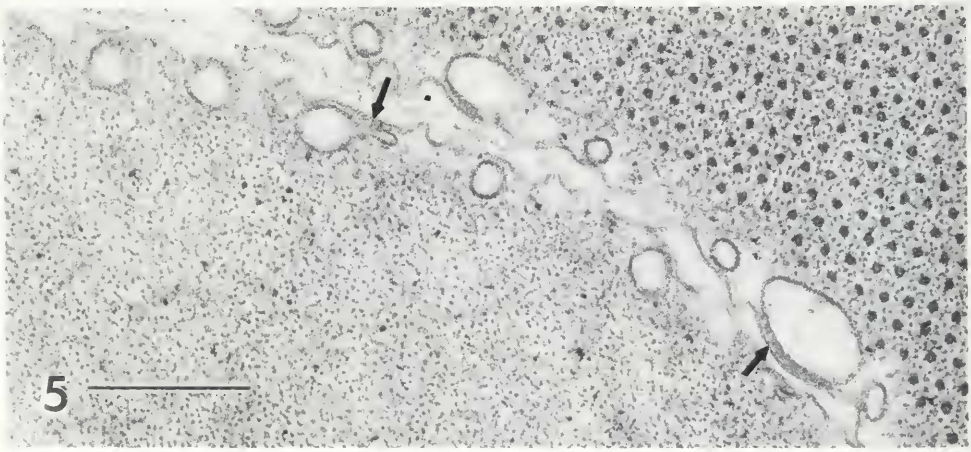

FIGURE 5. Cross-section of striated muscle cells showing the surface couplings (arrows) of the element of the sarcoplasmic reticulum to the cell surface. Scale = 0.5 μm.

Smooth muscle adductor

The fusiform cells of the smooth muscle adductor are about 10 μm at their widest diameter and contain one elongated nucleus per cell located in a peripheral position at the widest point. There are no indentations of the cell surface to form a T-system (Fig. 6). The sarcoplasmic reticulum consists of vesicles and irregularly shaped tubes or channels that connect the vesicles forming a complicated network just under the cell surface (Figs. 7–9). Fewer channels fuse with each vesicle than is the case in the striated muscle and the vesicles are slightly larger and more irregular in outline than those observed in the striated muscle. They range in width from 0.12 to 0.2 μm and in length up to 0.5 μm with ribosomes often attached. In transverse sections, surface couplings can be seen between the walls of the vesicles and the cell surface (Fig. 9). The tubes in the smooth muscle are uneven in bore, ranging from 15 to 40 nm. A filamentous coating is associated with the luminal wall of both the

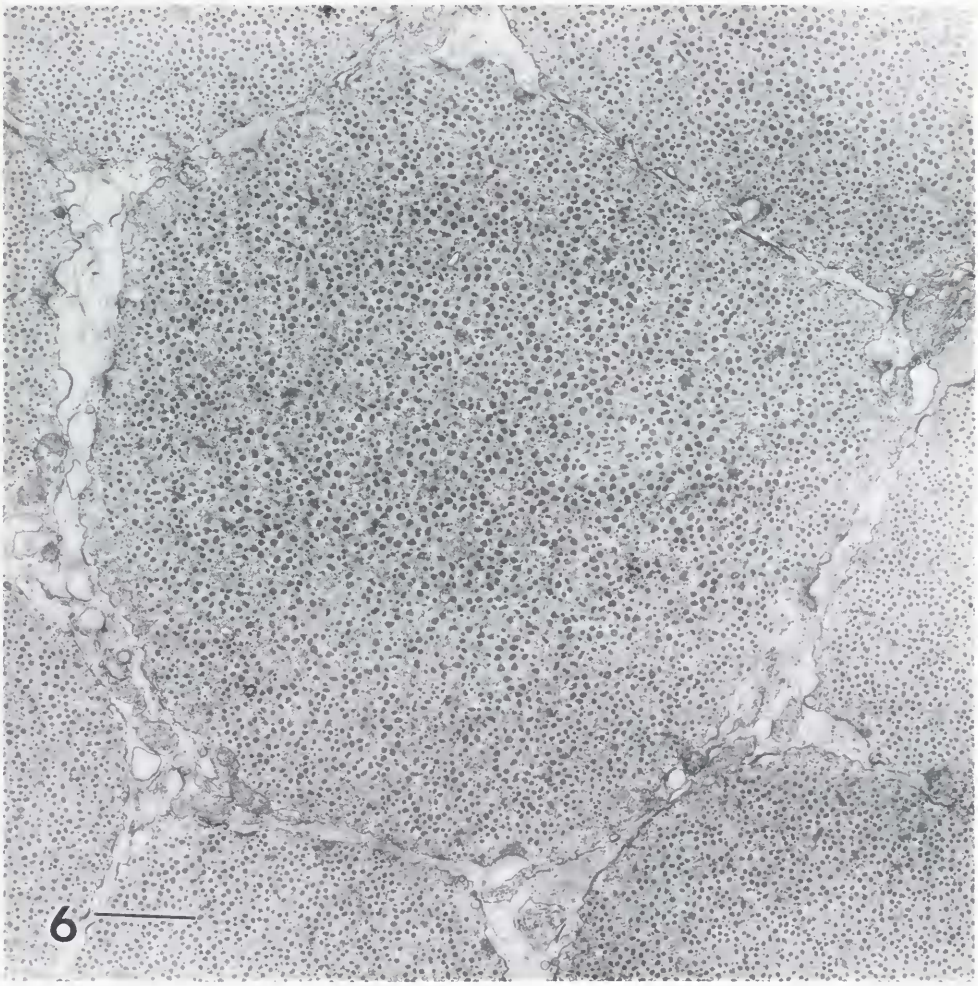

FIGURE 6. Transverse section of the smooth muscle adductor. Scale = 1 μm.

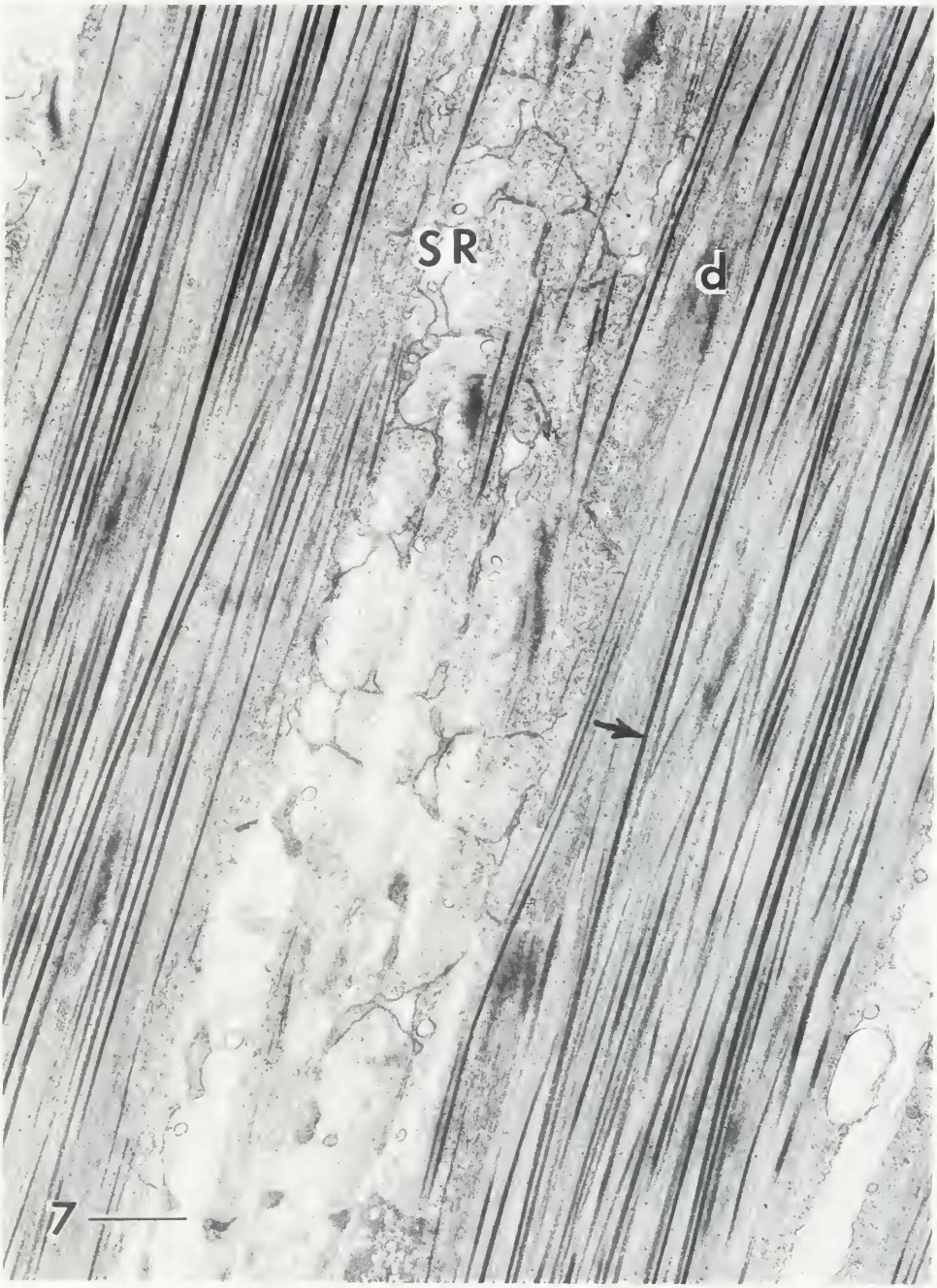

FIGURE 7. Longitudinal section of the smooth adductor illustrating dense bodies (d), thick filaments (arrow), and elements of the sarcoplasmic reticulum (SR). Scale = 1 μm.

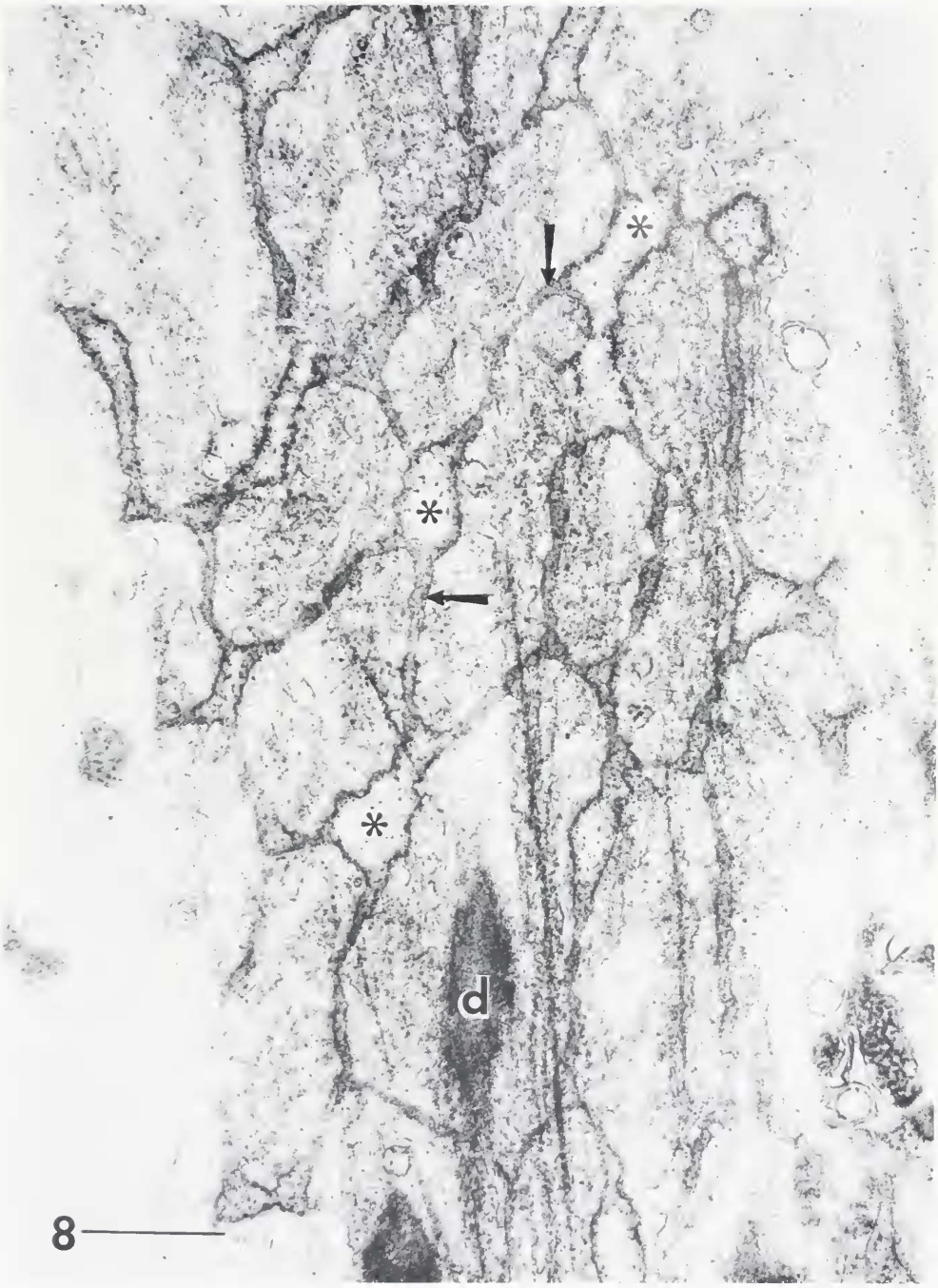

FIGURE 8. Tangential section of the wall of a smooth muscle cell demonstrating dense bodies (d) and the interconnections of the terminal cisternae (asterisks) via irregular tubes (arrows). Scale = 0.5 μm.

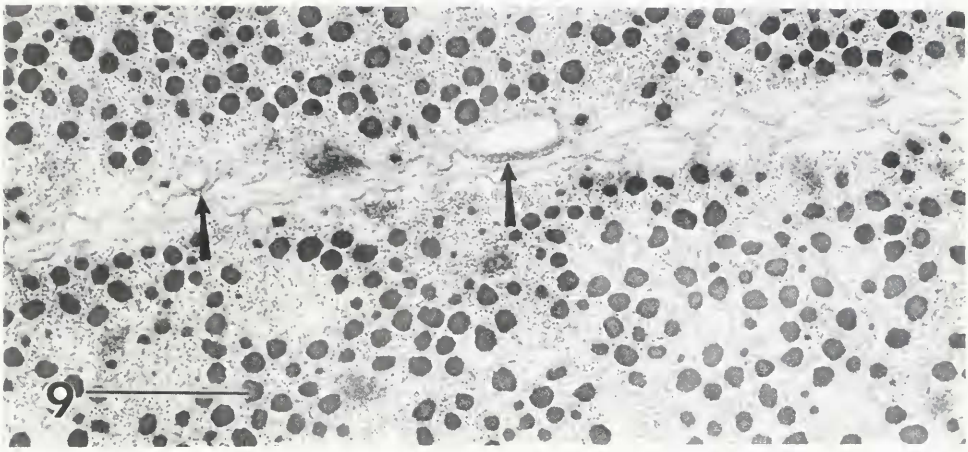

FIGURE 9. Cross-section of the smooth adductor with surface coupling between vesicles of the sarcoplasmic reticulum (arrows) and the cell surface. Scale = 0.5 μm.

vesicles and tubes of the sarcoplasmic reticulum. The area occupied by both elements of the sarcoplasmic reticulum in smooth muscle cells was about ½% of the non-nuclear cross-sectional area of the smooth muscle adductor cell (average of 15 measurements).

DISCUSSION

In scallops, both cross-striated and smooth adductor muscles possess a lacy network of sarcoplasmic reticulum localized just under the cell surface (*cf.* Figs. 4 and 8). Both systems have flattened vesicles coupled to the surface cell membrane via short structures, *i.e.*, surface couplings (Franzini-Armstrong, 1972). In the two muscles the vesicles are interconnected via tubules. The uniform diameter of the cross-striated sarcoplasmic reticulum tubules is in contrast to the non-uniform bore of the smooth muscle sarcoplasmic reticulum. The alignment of the tubes is along the long axis of the myofilaments and cell fiber.

A comparison of the two muscles in cross-sections (*cf.* Figs. 1 and 6) and in tangential sections (*cf.* Figs. 4, 10 and 8, 11) reveals at first glance that there is much more sarcoplasmic reticulum in the cross-striated muscle than in the smooth muscle. Our measurements of the sarcoplasmic reticulum demonstrated that 4% of the cross-sectional area of the cross-striated muscle is occupied by sarcoplasmic reticulum while in smooth muscle it is only 0.5%. The average cell size of the scallop striated muscle cell has a width of 10 μm and a thickness of 1.5 μm (area = 15 μm^2) while the average diameter of a smooth muscle cell was 6 μm (area = 28 μm^2). Thus the striated cells have about half the cross-sectional area of a smooth muscle cell, but eight times as much sarcoplasmic reticulum, resulting in a sixteen-fold advantage for the cross-striated muscle over that of the smooth muscle. The abundance of sarcoplasmic reticulum in the cross-striated muscle provides a morphological basis for its fifty-fold faster contraction rate and 128-fold faster relaxation time (Prosser, 1973). This sixteen-fold factor may be even greater when the relative rates of biochemical pumping of calcium ions (ATPase) is eventually compared in these two muscles: striated *versus* smooth.

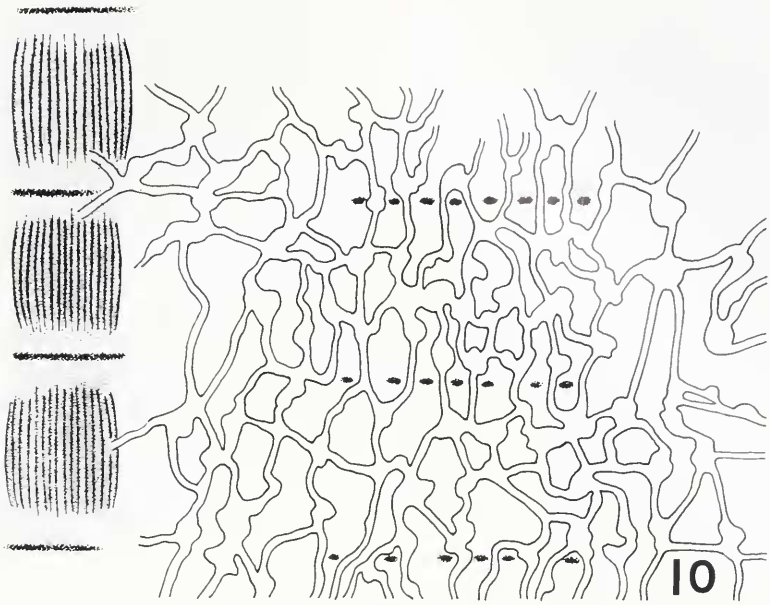

FIGURE 10. Diagram of a tangential section of a cross-striated adductor muscle cell.

The striated part of the adductor muscle is used for swimming and therefore, it is not surprising that it has a great deal more sarcoplasmic reticulum than the smooth muscle which is responsible for keeping the shell closed. The large amount of sarcoplasmic reticulum in the cross-striated muscle as compared to the smooth muscle of the scallop adductor corresponds to the general relationship observed among vertebrate muscles where the faster contracting muscles have a more elaborate and greater quantity of sarcoplasmic reticulum than slower contracting muscles (Franzini-Armstrong, 1972).

In vertebrates, mononuclear presumptive myoblasts fuse with one another to form a large multinucleated cell (reviewed by Fischman, 1972). In the striated scallop adductor, fusion of myoblasts evidently does not occur as evidenced by the absence of multinucleated cells and the presence of junctions connecting the ends of the muscle cells. These junctions resemble the intercalated discs of vertebrate and invertebrate cardiac muscle (reviewed by Sanger, 1979). Similar junctions were also observed in the striated adductor muscle cells of the scallop, *Aequipecten irridians* (Sanger, 1979; Nunzi and Franzini-Armstrong, 1981). In contrast to vertebrate cross striated muscles, there are no invaginations of the cell surface of the scallop cross-striated muscle surface to form a transverse tubular system. The scallop adductor muscle cells are small enough that diffusion of calcium from the cell surface to the sarcoplasmic reticulum can occur without the need for a transverse tubular system. In effect, a single scallop cross-striated muscle cell corresponds to one myofibril in vertebrate skeletal muscle (Sanger, 1971).

Vertebrate smooth muscle has much less sarcoplasmic reticulum than vertebrate striated muscle (Devine *et al.*, 1973) and does not have a transverse tubular system. There are a group of surface invaginations about 0.3 μm deep (caveolae) which are closely associated with the loose network of sarcoplasmic reticulum. Surface couplings have been observed between the cell surface and peripheral elements of the

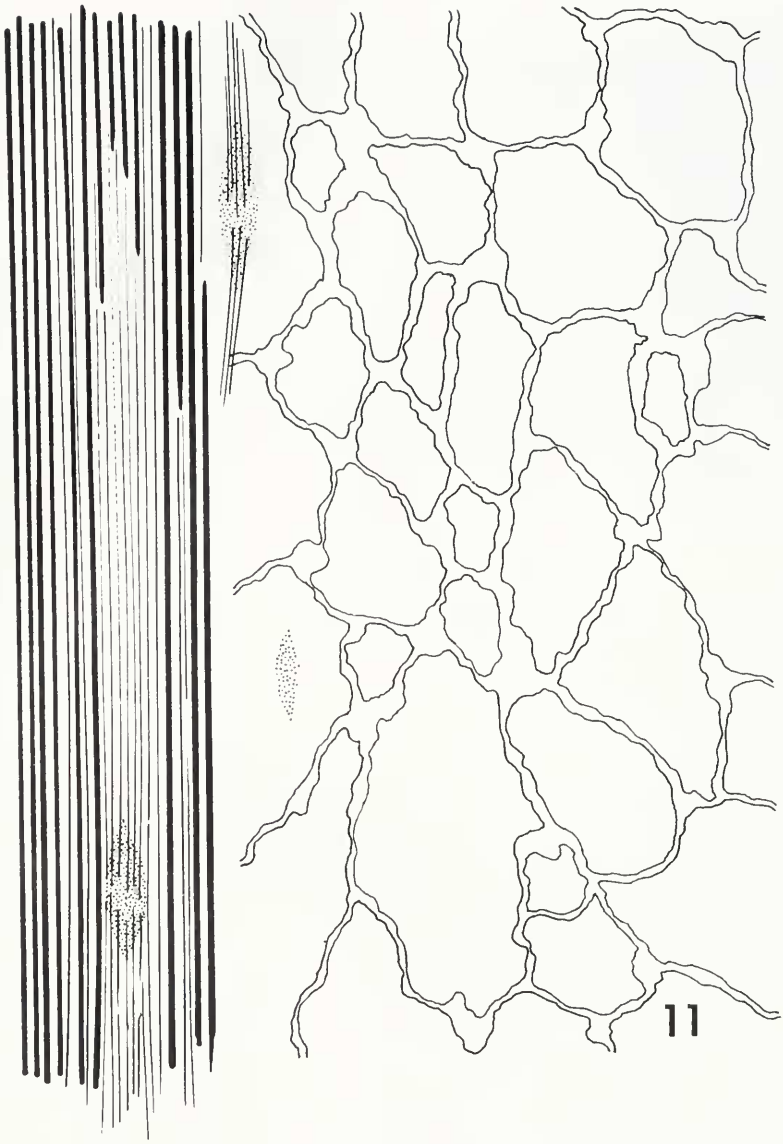

FIGURE 11. Diagram of a tangential section of a smooth adductor muscle cell.

sarcoplasmic reticulum but not between the sarcoplasmic reticulum and the caveolae. In some vertebrate smooth muscles, the sarcoplasmic reticulum consists of flattened vesicles interconnected with tubules of irregular diameter (Fig. 2 of Devine *et al.*, 1973). The pattern is similar to that observed in the scallop smooth muscle.

The scallop smooth adductor muscle like its cross-striated counterpart lacks any surface invaginations. These smooth muscle cells, while larger than the striated muscle cells, contract much more slowly than the striated cells (Prosser, 1973) and thus do not really need surface invaginations. Almost all invertebrate smooth muscle cells lack any surface indentations corresponding to vertebrate smooth muscle

caveolae. Sarcolemmic invaginations in smooth muscle were observed in a few molluscan cells (Sanger and Hill, 1972; Prescott and Brightman, 1976). The best documented case is the radula protractor muscle of the whelk, *Busycon canaliculatum* (Sanger and Hill, 1972, 1973). This muscle exhibited an extensive system of tubular invaginations of the cell surface which were termed sarcolemmic tubules. The tubules, 60 nm in diameter and 0.5 μm in length, were limited to just under the cell surface. Moreover two other smooth muscles (blood vessel and epineural smooth muscle) from the same animal also possessed these surface invaginations (Sanger, 1973). No surface couplings were observed between the sarcolemmic tubules and the sarcoplasmic reticulum (Sanger and Hill, 1972). The significance of these surface specializations with regard to the functions of the smooth muscle is unknown.

Whether the sarcoplasmic reticulum plays a role in the catch properties of the scallop smooth muscle is not known. There does not appear to be any unusual morphological entity present in the sarcoplasmic reticulum of the smooth muscle other than the uneven nature of the bore of the tubular elements in the catch muscle. However until some biochemical analysis is done on the two different sarcoplasmic reticulum systems it is difficult to see what effect this could have on the physiological behavior of the muscles.

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